SPECTROPHOTOMETRIC ANALYSIS AND EVALUATION OF SUSTAINED RELEASE OF 2 % CHLORHEXIDINE FROM A POLYMERIC MEDIUM (PVA & PVA + AgNPs) AND ITS EFFICACY AGAINST E. FAECALIS – AN INVITRO STUDY

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DECLARATION

TITLE OF DISSERTATION	**SPECTROPHOTOMETRIC ANALYSIS AND EVALUATION OF SUSTAINED RELEASE OF 2% CHLORHEXIDINE FROM A POLYMERIC MEDIUM (PVA & PVA + AgNPs) AND ITS EFFICACY AGAINST E.FAECALIS – AN INVITRO STUDY**
PLACE OF THE STUDY	**Tamil Nadu Government Dental College & Hospital, Chennai – 3.**
DURATION OF THE COURSE	**3 YEARS**
NAME OF THE GUIDE	**DR. M. KAVITHA.**
HEAD OF THE DEPARTMENT	**DR. M. KAVITHA**

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CONTENTS

INTRODUCTION

Microorganisms play a fundamental role in the etiology of pulp and periapical diseases. Their control and elimination are important during endodontic treatment[24]. Unlike primary endodontic infections, which are polymicrobial in nature and dominated by gram-negative anaerobic rods, the microorganisms involved in secondary infections are composed of one or a few bacterial species[15,64].

Enterococcus faecalis is more likely to be found in failed cases than in primary infection[66]. It is commonly found in a high percentage of root canal failures and it is able to survive in the root canal as a single organism or as a major component of the flora[9]. Studies investigating its occurrence in root-filled teeth with periradicular lesions have demonstrated a prevalence ranging from 24 to 77%[41,42,47]. Starvation increases the resistance of E. faecalis 1000-fold to 10,000-fold[50]. It is probable that the physiologic state of the cells, particularly in retreatment cases, is closest to the starvation phase[69].

Cleaning and shaping of the root canal reduce the bacterial population but do not completely eliminate them. One possible reason for persistent

endodontic infection might be due to the retention of microorganisms in the dentinal tubules of the root canal[54] as a result of the anatomic complexity and diversity of root canals, as well as the subsequent limitations in access by instruments and irrigants[46,27,57]. Hence, the use of an intra canal medicament helps in the elimination of bacteria that remain even after cleaning and shaping, thereby providing an environment conducive for periapical tissue repair[6].

Calcium hydroxide is the most widely used intra canal medicament, requiring a disinfection period of 7 days[66]. The high pH of calcium hydroxide formulations alters the biologic properties of bacterial lipopolysaccharides in the cell walls of gram-negative species and inactivates membrane transport mechanisms, resulting in bacterial cell toxicity[63]. However, several studies demonstrated that $Ca(OH)_2$ fails to eradicate Enterococcus faecalis residing in infected root canal systems[14].

An explanation for the resistance against $Ca(OH)_2$ might be the ability of E. faecalis to invade dentinal tubules, isthmuses and other ramifications of a root canal system. Furthermore, it has been documented to be able to survive for prolonged periods in high alkalinity and harsh nutrient conditions[14]. Evans et al. (2002) reported that the survival of E. faecalis at high pH was due to the functioning of a proton pump with the capacity to acidify cytoplasm. On the other hand, the buffering capacity of dentin can inhibit the pH increase and the antimicrobial activity of $Ca(OH)_2$ in the root canal[41]. The search for a better alternative has lead to the introduction of newer

antimicrobial agents like chlorhexidine, metronidazole, and particulate bioactive glass as intracanal medicament.

In vitro studies have indicated that chlorhexidine may be potent in the elimination of E. faecelis from the root canal system[14]. It is active against a wide range of microorganisms, such as Gram positive and Gram-negative bacteria[12]. CHX has an antibacterial efficacy comparable to that of sodium hypochlorite (NaOCl)[39]. In addition, it is also effective against strains resistant to $Ca(OH)_2$. 2% CHX has been proved to be an efficient agent against E. faecalis[21].

In addition to its immediate action on bacteria, chlorhexidine can be adsorbed onto and subsequently released from dental tissues, resulting in substantive antimicrobial activity or "substantivity"[2,23]. Such substantivity has been shown in vitro in root canal medicated with chlorhexidine by using different vehicles (i.e liquid, gel or controlled release devices CRD).

The rationale for using sustained release systems for intracanal medication is that it is necessary to sterilize the root canal system and to maintain its sterility throughout the treatment. It follows, therefore, that the medication should remain active at a constant concentration for a designated time period. In addition, if the antibacterial medicament has an affinity for dentine and will be slowly released from it, this in turn will prolong the period of antimicrobial activity.

Hydrogels are gaining increasing popularity in the area of controlled-release drug delivery[43]. These polymers are generally glassy in the dehydrated state but swell to become an elastic gel upon water penetration. The entrapped drug within the swelling matrix concomitantly dissolves and diffuses through the swollen network into surrounding aqueous environment. The rate of drug release from hydrogels is regulated by crosslinking density and the extent of swelling[43].

Poly (vinyl alcohol) (PVA) is a hydrophilic polymer with properties of forming hydrogel. It absorbs water, swells forms and it has extensively been used in controlled-release applications[44]. It has been used as a controlled drug delivery system for rectal propranolol, atenalol, indomethacin, phenylpropanolamine and emedastin/HCI[36].

Previous studies have shown that polyvinyl alcohol has good compatibility with chlorhexidine digluconate and has been used as drug carrier in stomatological dressings[35,48]. However, none of the studies have utilized polyvinyl alcohol as a drug carrier for intracanal medicament.

In this study we have analyzed the release kinetics of 2 % CHX from PVA hydrogel and studied the efficacy of this sustained release mechanism against E faecalis in vitro.

AIMS AND OBJECTIVES

a. To synthesize Polyvinyl alcohol + 2 % Chlorhexidine matrix and Polyvinyl alcohol + Silver nanoparticles + 2 % Chlorhexidine matrix.
b. Analysis of the sustained release of 2% chlorhexidine from Poly vinyl alcohol hydrogel.
c. In vitro Evaluation of the efficacy of this sustained release mechanism against E faecalis by measurement and comparison of the zone of inhibition over a period of a week.

REVIEW OF LITERATURE

Enterococcus faecalis & Intra canal Medicaments:

The literature is teeming with immense studies on the use of various medicaments for the disinfection of the root canal and their efficacy against E. Faecalis.

Haapasalo et al. [14] **(1987)** tested Camphorated paramonochlorophenol (CMC) and a calcium hydroxide compound, Calasept, for their disinfecting efficacy toward E. faecallis – infected dentin. Liquid CMCP rapidly and completely disinfected tubules, whereas CMCP in gaseous form disinfected tubules less rapidly. Calasept failed to eliminate, even superficial E. faecalis in the tubules.

Chong et al.[6] **(1992)** stated that intra canal medicaments should only be used for root canal disinfection as part of controlled asepsis in infected root canals, and their role is secondary to cleaning and shaping of the root canal. Thorough canal debridement and adequate canal preparation are more pertinent and their importance is emphasized. Bacteriological sampling may be necessary if a tooth does not respond to treatment, to help in the choice of intra canal medicament.

Gomes et al.[13] **(1996)** studied variation in the susceptibilities of endodontic microflora to chemical procedures and confirmed that organisms like E. faecalis were recovered from canals after thorough Bio Mechanical Preparations. He concluded certain organisms like E. faecalis are difficult to be eliminated from infected root canals.

Sundqvist et al.[69] **(1998)** conducted a study to determine what microbial flora was present in teeth after failed root canal therapy and to establish the outcome of conservative re-treatment. They concluded that the microbial flora in canals after failed endodontic therapy differed markedly from the flora in untreated teeth. Infection at the time of root filling and size of the periapical lesion were factors that had a negative influence on the prognosis. Three of four endodontic failures were successfully managed by re-treatment.

Bettina Basrani et al.[2] **(2002)** conducted a spectrophotometric analysis using (1) 2% chlorhexidine (CHX) gel, (2) 0.2% CHX gel, (3) 2% CHX solution, (4) Ca(OH)2, (5) Ca(OH)2+ 0.2% CHX gel, (6) 2% CHX solution + a 25% CHX-containing controlled-release device, (7) saline and (8) gel vehicle. After medication for 7 days , the authors concluded that canal dressing for 1 week with 2% CHX gel may provide residual antimicrobial activity against E. faecalis.

Isabelle Portenier et al.[19] **(2003)** Stated that in endodontics, E. faecalis is rarely present in primary apical periodontitis, but it is the dominant microorganism in rootfilled teeth presenting with post-treatment apical periodontitis. It is often isolated from the root canal in pure culture, but it can also be found togetherwith some other bacteria or yeasts. While there is no doubt about the pathogenicity of E. faecalis in endodontic infections, it seems to be rarely associated with acute infections and flare-ups. Eradication of E. faecalis from the root canal remains a challenge, while chlorhexidine and combinations of disinfectants show some promise.

Vivacqua-Gomes et al.[71] **(2005)** showed that neither single- nor multiple-visit root canal treatment ex vivo, eliminated E. faecalis completely from dentinal tubules. Up to 60 days after root filling, E. faecalis remained viable inside dentinal tubules. When no sealer was used, E. faecalis presented a higher growth rate.

Kayaoglu et al.[25] **(2005)** concluded that a minor increase in pH up to 8.5, which may be a consequence of insufficient treatment with alkaline medicaments such as calcium hydroxide, increases the collagen-binding ability of E. faecalis, in vitro. This can be a critical mechanism by which E. faecalis predominates in persistent endodontic infections.

Sustained release of Chlorhexidine

Friedman M et al.[10] **(1985)** studied the effectiveness of a sustained-release delivery system in the form of orthodontic appliances coated by ethyl cellulose polymer for chlorhexidine release in plaque prevention. They concluded that the conditions in the oral cavity and the formulation used did not facilitate such prolonged prevention of plaque accumulation. However, it may be assumed that by altering the film components and method of preparation (i.e., initial drug concentration and film thickness applied), it will be possible in clinical use, too, to sustain the necessary level of chlorhexidine release for longer periods. Such a delivery system could be a treatment of choice for partial-denture- and orthodontic-appliance-wearers.

D.B. Mirth et al.[7] **(1989)** prepared Copolymers of hydroxyethyl methacrylate (HEMA) and methyl methacrylate (MMA) to fabricate a membrane- controlled reservoir-type controlled-release delivery system for chlorhexidine for intra-oral use. The chlorhexidine released on day 30 was biologically active, as determined by a serial dilution assay against Streptococcus mutans.

L Heling et al.[32] **(1992)** evaluated the effect of chlorhexidine in solution and in a sustained-release device as an intra canal medication. The intra canal medicaments tested were 0.2%chlorhexidine gluconate solution

(CHX), chlorhexidine in a sustained-release device (1.2 mg) (SRD), camphoratedparamonochlorophenol (CMCP), and a control (no medication).Each medicament was introduced into the lumen of infected dentine specimen and Incubated for 5 min. 24 h, 48 h or 7 days at 37°C. The bacteriological samples were taken and were collected in test-tubes containing growth medium and incubated for 24 h. The optical density of the medium was recorded by means of a spectrophotometer at a wavelength of 540 nm. There was a statistically significant difference between the control group and all the medicaments tested.

L Heling et al.,[31] **(1992)** evaluated the efficacy of the antibacterial activity of $Ca(OH)_2$ and a sustained-release device containing chlorhexidine (SRD) in both sterlization and prevention of secondary infection of the root canal system. The degree of bacterial infection of the root canal was tested after incubation periods of 24 h, 72 h and 7 days with the medicaments. Their efficacy in preventing secondary infection after recontamination was tested after 72 h and 7 days. The results demonstrated that both formulations of the SRD significantly reduced the bacterial population in the primary infected groups, as well as preventing secondary infection of the dentinal tubules in the recontaminated group. By contrast, Ca(OH)2 did not show any

antibacterial activity, and failed to sterilize the dentinal tubules or prevent secondary infection after recontamination at the time periods examined.

Senel S et al. [56] **(2000)** designed a formulation containing Gels (at 1 or 2% concentration) or film forms of chitosan containing 0.1 or 0.2% CHX and their in vitro release properties were studied. The antifungal activity of chitosan itself as well as the various formulations containing CHX was also examined. Release of CHX from gels was maintained for 3 h. A prolonged release was observed with film formulations. The highest antifungal activity was obtained with 2% chitosan gel containing 0.1% CHX.

Peter N. Galgut [45] **(2001)** made a reference for the slow release preparation of Chlorhexidine for professional application into periodontal pockets containing 2.5 mg of chlorhexidine digluconate in a hydrolysed gelatin base. When placed in periodontqal pockets, the gelatin base degrades and releases the chlorhexidine over a 7 to 10 days period. The sustained release of the antimicrobial over a prolonged period of time provided an enhanced disinfection of the pockets in which it is placed.

Shaul Lin Ofer Zuckerman[60] **(2003)** used 5% chlorhexidine and slow-release device (Activ Point) for 7 days and placed it inside 9 infected root canals, in another nine canals irrigation with 10 ml of 0.2% chlorhexidine was used, and the remaining nine served as positive control.

Powder dentin samples obtained from within the canal lumina were examined for the presence of vital bacteria. Heavy bacterial infection was observed at the layer close to the lumen in the control specimens, decreasing rapidly from layer to layer up to the deepest layer tested (400-500 μm), which contained several hundred colony forming units. Viable bacteria in each layer of dentin were significantly reduced with chlorhexidine irrigation solution and were completely eliminated with the chlorhexidine slow-release device.

Palmer G et al[40] **(2004)** investigated the use of an experimental GIC as a carrier for the release of chlorhexidine acetate (CHX) at included concentrations ranging from 0.5% to 13.0% of CHX by weight. Release into water was examined using high-performance liquid chromatography. All measurable chlorhexidine was released within 22 h1/2, however this was less than 10% of the total mass incorporated in the specimens. An increased percentage of CHX incorporated into the powder gave an increased release into the surrounding water. The bulk of the CHX was retained within the cement. In order to explore the effect of CHX -inclusion on the cement properties, compressive strengths, working and setting times were also measured. In general, compressive strengths were found to be decreased in

direct proportion to the quantity of CHX added, while working and setting times increased.

Cetin EO et al.[5] **(2004) prepared** Cellulose acetate films containing chlorhexidine gluconate, indomethacin, and meloxicam and cut in a form to fit to the periodontal pocket anatomy. The release of active agents was studied in 10 ml artificial saliva at 37 degrees C. Determinations were carried out spectrophotometrically and the formulations showed two different release patterns for a total observation period of approximately 120 hrs. When the formulations of the three active agents were compared, the release patterns of meloxicam and chlorhexidine gluconate were found to be similar, while the indomethacin-containing formulation exhibited the fastest release rate.

Spangberg[67] **(2005)** evaluated the suitability of using chitosan, poly (lactide glycolide acid) (PLGA), and polymethyl methacrylate (PMMA) to control the release of chlorhexidine digluconate (CHX) from a prototype of controlled release drug device for root canal disinfection. The result showed that release rate of CHX was the greatest in the noncoated group, followed by the chitosan-coated group, the PLGA-coated group, and the PMMA-coated group. Pores were observed on the surface of the prototypes that were

coated with PLGA and PMMA. When the pore size was smaller, the release rate was lower.

K.J. Anusavice et al [23] **(2006)** tested the hypothesis that the release of chlorhexidine from a urethane dimethacrylate and triethylene glycol dimethacrylate resin system can be effectively controlled by the chlorhexidine diacetate content and pH. The filler concentrations were 9.1, 23.1, or 33.3 wt%, and the filled resins were exposed to pH 4 and pH 6 acetate buffers. The results showed that Fickian diffusion was the dominant release mechanism. The rates of release were significantly higher in pH 4 buffer, which was attributed to the increase of chlorhexidine diacetate solubility at lower pH. The higher level of filler loading reduced the degree of polymerization, leading to a greater loss of organic components and higher chlorhexidine release rates.

Yoon Lee, DDS et al.[73] **(2008)** tested the following intracanal medicaments in infected root canals: calcium hydroxide, a polymeric chlorhexidine-controlled release device (PCRD), a polymeric controlled release device without chlorhexidine (CHX), 0.2% CHX solution, and sterile saline. Dentin samples (at 200 μm and 400 μm depths) were collected from the medicated canal lumens after 1 week of medication and placed in growth medium. Bacterial growth was assessed spectrophotometrically. The OD

values at both depths were significantly lower in the PCRD group than in the other experimental groups. These results indicate that a PCRD can be an effective intracanal medicament against E. faecalis.

Pragati S et al.[51] **(2009)** showed that fibers containing 20% (v/v) chlorhexidine, when placed into periodontal pockets, exhibited a prompt and marked reduction in signs and symptoms of periodontal disease. To retard drug release, drug-impregnated monolithic fibers were developed by adding drug to molten polymers, spinning at high temperature and subsequent cooling. Several polymers such as poly (e-caprolactone) (PCL), polyurethane, polypropylene, cellulose acetate propionate and ethyl vinyl acetate (EVA) have been investigated as matrices for the delivery of drug to the periodontal pocket. Sustained release devices composed of cross-linked fish gelatin (bycoprotein) containing chlorhexidine diacetate or chlorhexidine hydrochloride have been developed by Steinberg. Films based on synthetic biodegradable polymers such as poly (lactide-co-glycolide) (PLGA) containing tetracycline have been developed for modulated-release of drug in the periodontal pocket as slab like device.

Raso, Eliete et al.[53] **(2010) p**repared and characterized a controlled release system based on porous silica loaded with chlorhexidine (CHX) to evaluate its antimicrobial activity. The kinetics release parameter of the drug

showed that the CHX systems release profile followed zero order release until 400 h after the burst effect at the first 8 hrs. Chlorhexidine therapeutic range was reached near first hour for all systems. The chlorhexidine porous silica system was biologically active against *Enterococcus faecalis* and *Candida albicans* in vitro. The systems showed an efficient CHX controlled release modulated by the presence of the β-cyclodextrin and by the porous silica matrices, providing effective antimicrobial activity.

Simchuer Wilaiwan et al [62] **(2010)** prepared silk fibroin (SF)/gelatin (G) hybrid films by a solvent evaporation method for loading chlorhexidine diacetate (CHX). The SF and G solution in different ratios were mixed with CHX and placed on the 5 cm polystyrene plates before drying to obtain hybrid films. It was found that the CHX released from the SF film in higher rate than hybrid and G films. Polarity, flexibility as well as component ratio of each polymer play important role on the releasing of CHX.

Racheli Ben-Knaz[52] **(2010)** demonstrated the entrapment of chlorhexidine digluconate (CHD) within an aggregated silver matrix, a metal known for its own biocidal qualities, forming the CHD withsilver composite. The bactericidal efficacy against E. coli is evaluated and compared with the separate components. While the bactericidal efficacy of the individual ingredients (CHD and metallic silver) is very low, CHD with silver exhibits

a markedly enhanced efficacy. This enhanced bactericidal effect is partially attributed to the simultaneous release and presence of the active biocidal ingredients CHD and Ag*R* in the solution.

Poly Vinyl Alcohol as sustained releasing agent

Gay, M. H et al[34] **(1987)** evaluated wound dressings based on polyvinyl alcohol, polyhydroxyethyl methacrylate, polyacrylamide and polyethylene oxide. He found that release rates are dependent upon water content, degree of hydrogel cross linking, concentration of plasticizer, polymer molecular weight, degree of hydrolysis of polyvinyl alcohol, and solubility of the antimicrobial agent. Polyvinyl alcohol based hydrogels containing either tetracycline free base or chlorhexidine diphosphanilate were efficacious in-vivo in a wound model with an established Streptococcus pyogenes infection. In addition chlorhexidine diphosphanilate hydrogels were efficacious against established Staphylococcus aureus infections and mixed infections containing both organisms.

Bruno Gander[3] **(1989)** evaluated three types of polyvinyl alcohol which were cross linked by glutaraldehyde to form water swellable materials possessing a three dimensional molecular network. Proxyphylline and theophylline were incorporated into the polymer networks during the cross linking reaction. Drug release from the highly cross linked gels could be

controlled over more than 12 hr, as the diffusion process in these very dense macromolecular networks is rather slow. The extent of branching and entanglement of the polymer chains appeared to have an important effect. In addition, the release was influenced greatly by the amount and, to a lesser extent, by the type of drug in the network.

Kazuhiro Morimoto et al.[26] **(1990)** evaluated a bunitrolol preparation using poly(vinyl alchol) (PVA) hydrogel for hypertension as a transdermal delivery system. The release of bunitrolol from PVA hydrogel followed with Fickian diffusion. Longer freezing times, higher polymerization and higher concentration of PVA resulted in lower permeationand release. The plasma concentration of bunitrolol after application of hydrogel preparation onto the abdominal skins was relatively high at early times and sustained a plateau level during 48 hrs in rates.

Pluta J et al.[49] **(2001)evaluated** Dressings made from polyvinyl alcohol (PAV) and Hydroxypropylmethylcellulose (HPMC) with the addition of glycerol (GLY). The research on the release speed which is applied in topical therapy of chlorhexidine digluconate antiseptic in biopharmaceutical model proved the existence of close to rectilinear relation between the amount of released substance and release time for selected formulations.

Pluta J et al. [48] **(2002)**. They revealed that depending on the degree of polyalcohol hydrolysis, the dressings made on the basis of PVA and methylcellulose (MC) with the addition of hydrophilic substances were characterized by distinct rheological properties and released the active substance in various ways. The studies on the kinetics of the release of Chlorhexidine Digluconate, in biopharmaceutical model provided evidence of close to rectilinear relationship between the amount of released substance and the time of release for selected formulations.

Jaleh Varshosaz et al.[20] **(2002)** investigated the effect of drug release (theophylline) from Cross-linked poly {vinyl alcohol) (PVA) polymeric network . Changes in glutaraldehyde percentage (or cross-linking density) affected the swelling of the films. However, increasing PVA percentage caused more swelling. Drug loading efficiency was higher in gels with higher glutaraldehyde, PVA and theophylline percentages. Increasing contents of PVA and theophylline promoted the diffusion coefficient and drug release rate but glutaraldehyde had a reverse effect. The pH did not affect the swelling and diffusion coefficient. Water transport and drug release mechanism predominantly followed a Fickian model. It may be concluded that by changing the PVA structural parameters, a rate-controlled drug release is obtained.

Pragati et al[51] **(2009)** studied the distinguishable films composed of poly vinyl alcohol (PVA) and carboxymethyl-chitosan (CMCS) prepared by blending/ casting methods, and loaded with ornidazole as a periodontal drug delivery system. The blended films were found to be biocompatible, showed pH-responsive swelling, had a good retention at the application site and maintained high drug concentration at least for five days.

MATERIALS AND ARMAMENTARIUM

MATERIALS:

- Poly (vinyl alcohol) (PVA) (Fluka)
- Silver nitrate ($AgNO_3$) (Ranbaxy Lab. Ltd. India)
- Sodium Borohydride ($NaBH_4$) (Aldrich Chemicals)
- 20 % aqueous solution of Chlorhexidine Digluconate(Sigma Aldrich)
- Distilled water
- Phosphate buffered saline solution(pH 7.4)
- Enterococci faecalis (ATCC 29212)
- Sheep blood agar media (Highmedia)
- Peptone (Highmedia)

ARMAMENTARIUM:

- 100 mL flask
- Teflon coated magnetic stir bars
- Sonicator (Cole-Palmer)
- Electronic Weighing machine(Scaltec)
- UV spectrometer (Techcomp – 8500, UV-Vis)
- Vaccum dryer
- Microcentrifuge tubes
- Thermostat
- Hot air oven
- Incubator 37 ^{o}C
- Petri dishes
- Test tubes
- Laminar Flow
- Micropipette
- Scale
- Sterile gloves
- Mask
- Sterile Cotton Swab

METHODOLOGY FOR DRUG RELEASE:

Poly (vinyl alcohol) and Chlorhexidine Matrix Preparation:

Polyvinyl alcohol is a hydrophilic polymer and hence soluble in water. Ten gms of 72000 Daltons Molecular weight Poly vinyl alcohol with a degree of hydrolysis of 97.5 - 99.5 % was mixed in 100 mL of hot water at 80-90^{o}C to prepare 10 % solution of Polyvinyl alcohol[20]. After cooling the solution to room temperature, 10 mL of this solution was mixed with 10 mL of 2% Chlorhexidine digluconate solution. Chlorhexidine digluconate was purchased as 20% aqueous solution which was further diluted to 2% in the following manner.

20% Chlorhexidine = 20 gms in 100 ml of distilled water

= 2000 mgs in 100 ml of distilled water

= 20 mg in 1ml of distilled water

The required conc. of 2% Chlorhexidine is to be prepared from a solution of 20 mgs in 1 ml.

2% Chlorhexidine = 2 gms in 100 ml of distilled water

= 200 mgs in 100 ml of distilled water

= 2 mg / ml of distilled water

Therefore to convert a 20 mg/ml chlorhexidine solution to a 2 mg/ml solution, we diluted 1 ml of the concentrated 20 % solution with distilled water to get a 10 ml solution of 2 % Chlorhexidine solution.

9 ml of distilled water + 1ml of 20 % Chlorhexidine

= 10 ml of 2 % Chlorhexidine

Hence, 1 ml of 20 % aqueous solution of Chlorhexidine digluconate was measured using a micropippete and taken in a test tube, to this 10 ml of distilled water was mixed to make it 2% Chlorhexidine digluconate solution.

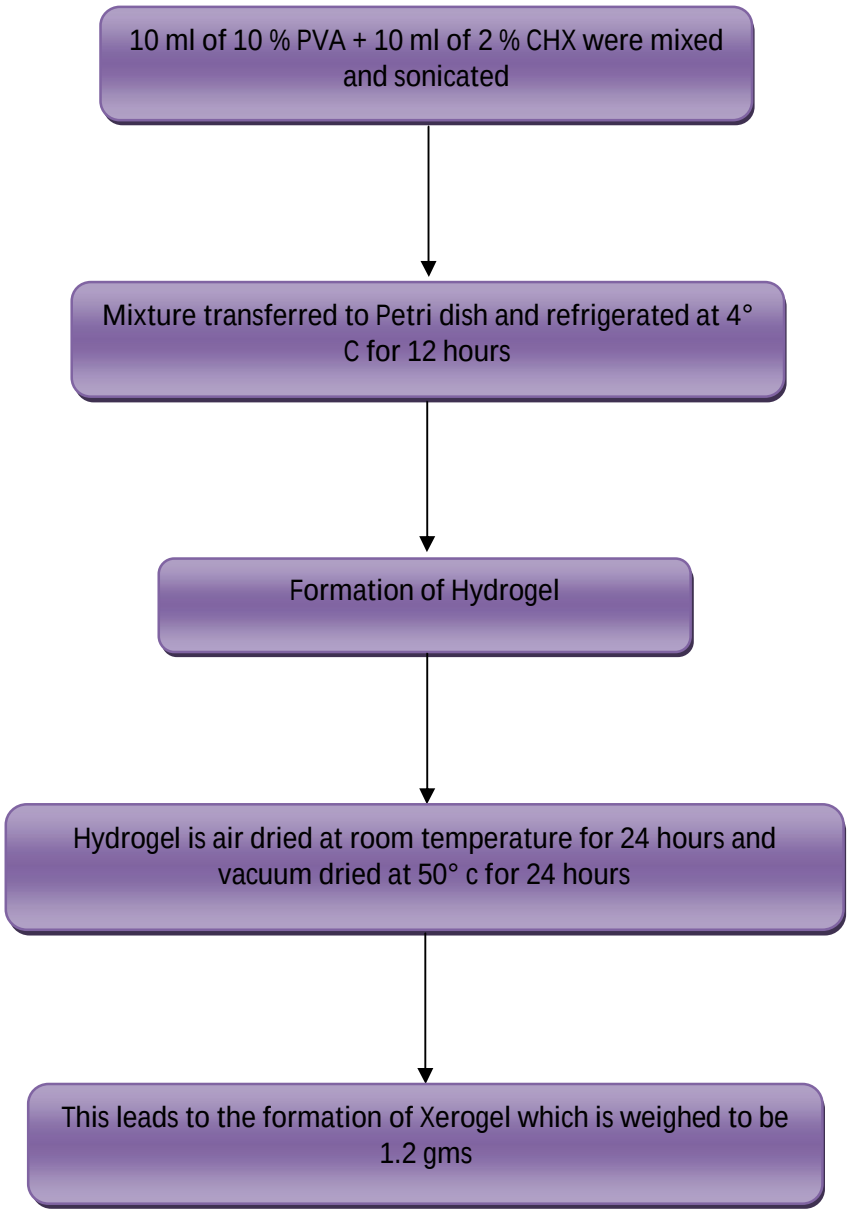
The mixture of 2 % Chlorhexidine digluconate and 10 % Polyvinyl alcohol which were taken in equal proportions (10 mL each), were sonicated thoroughly and poured into petri dishes. The petri dishes were sealed to prevent evaporation and stored in refrigerating conditions of 4 ^{o}C[28]. After 12 hrs, the hydrogel of Polyvinyl alcohol with the entrapped drug (2 % Chlorhexidine digluconate) was obtained. The cause of hydrogel formation by PVA is the cross linking between the polymer chains due to ionic interaction and hydrogen bonding [20] and imbibation of liquid into them.

Finally, the gel was air-dried at room temperature for 24 hrs followed by a vacuum-drying cycle at 50^{o}C for another 24 h until reaching a constant weight. When the water is removed from these swollen biomaterials they are called xerogels, which are the dried hydrogels[20].

This xerogel can be stored for a longer term. During the drying process of hydrogels, water evaporates from the gel and the surface tension of the water causes collapse of polymer chains and thus shrinking of the hydrogel body to only small fraction of its swollen size. Water absorption into this xerogel occurs by diffusion which is a very slow process leading to

very slow swelling. This slow swelling is used to slowly release loaded drug molecules. The weight of the xerogel was approximately 1.2 gm.

PVA + 2 % CHX Matrix Preparation

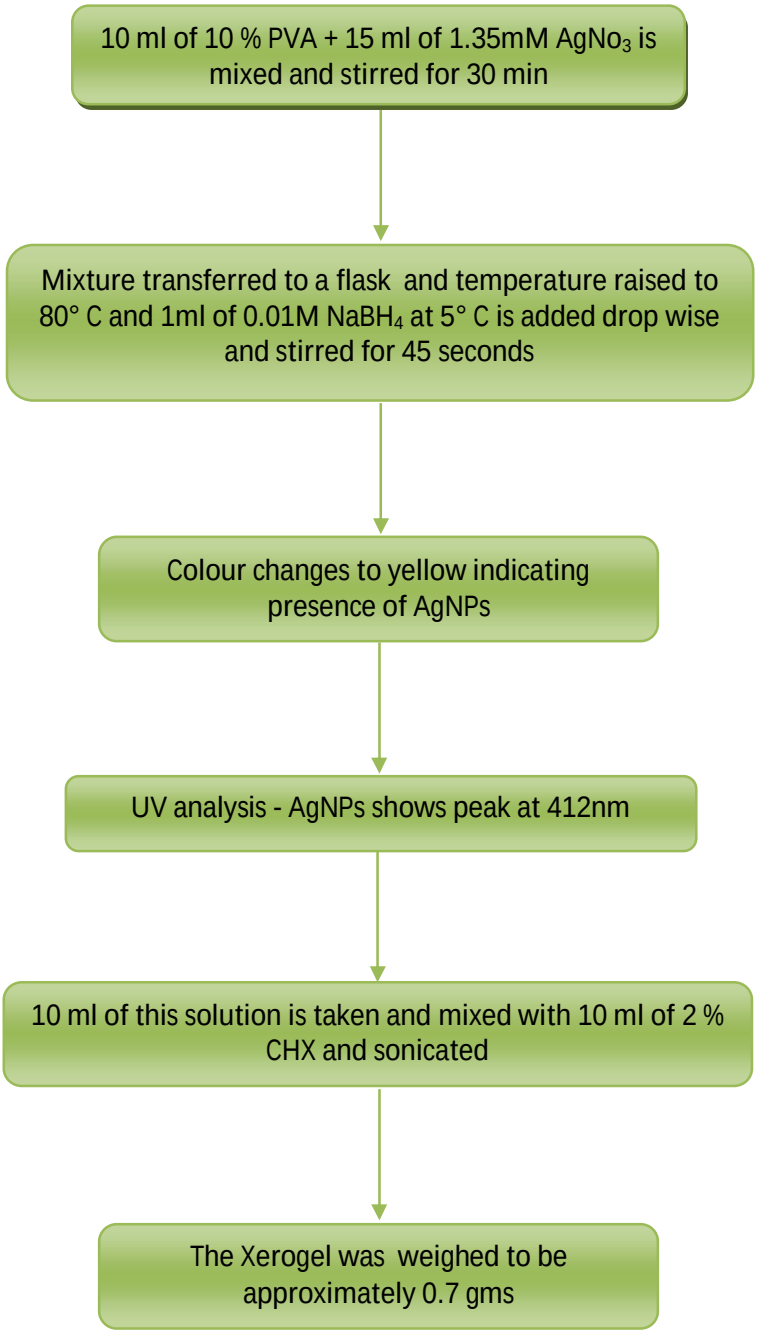


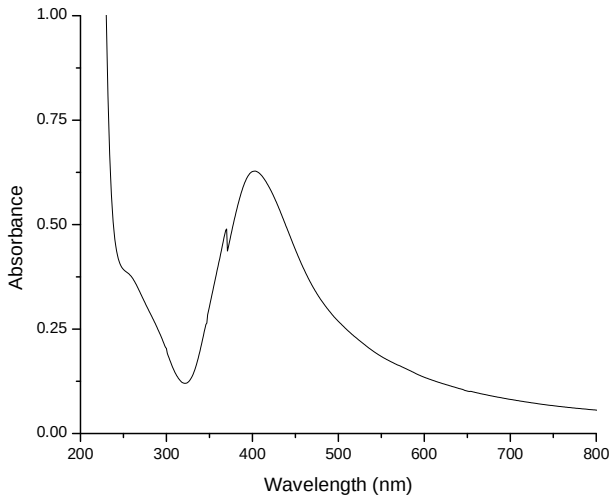
Synthesis of Polyvinyl alcohol stabilized Silver nanoparticles (AgNPs):

The Polymer (PVA) stabilized Silver nanoparticles are prepared using Sodium borohydride ($NaBH_4$) reduction in wet chemical method. The silver nitrate ($AgNO_3$) is the precursor and the stabilizer is polyvinyl alcohol.

A 100 mL flask is used for the synthesis of the nanoparticles. A stock solution of 1.35 mM $AgNO_3$ is prepared and sonicated to complete dissolution [30]. To the 15 mL of this solution, 10 mL of 10 % PVA solution is added and stirred for 30 min. The temperature of the solution is raised to 80^0C using a thermostat and 1 mL of 0.1M $NaBH_4$ solution (maintained under cold condition, 5^0C because hydrogen is volatile at higher temperature) is added dropwise with continuous stirring for 45 min. The colour of the solution changes to yellow indicating the presence of AgNPs. A UV spectrum of the solution after the nanoparticle formation was taken. Presence of the surface plasmon resonance peak at 412 nm confirms the formation of pure AgNPs. Now equal quantity (10 mL) of PVA stabilized silver nanoparticles and 2% Chlorhexidine digluconate were sonicated and casted in a similar manner as described above for PVA and Chlorhexidine digluconate until it reached a constant weight of 0.7 gm.

PVA +Ag NPs + 2 % CHXP matrix preparation





Plasmon resonance peak at 412 nm confirms the formation of pure AgNPs

Analysis of Drug release kinetics:

The release of chlorhexidine from polyvinyl alcohol is analysed with a simple experimental set up. The logic behind such an experimental set up is that release of drug from a hydrogel follows simple diffusion from a higher concentration to a lower concentration. In this experiment diffusion of the drug through a semi-permeable membrane (cellulose acetate membrane) was performed.

The semi-permeable membrane was soaked in phosphate buffered saline (PBS) which had pH of 7.4. Phosphate buffered saline is a water-based salt solution containing sodium chloride, sodium phosphate, and potassium chloride and potassium phosphate. It is available in powdered

form which can be mixed with appropriate amount of water to make a solution. The buffer helps to maintain a constant pH. The osmolarity and ion concentrations of the solution usually match those of the human body (isotonic).

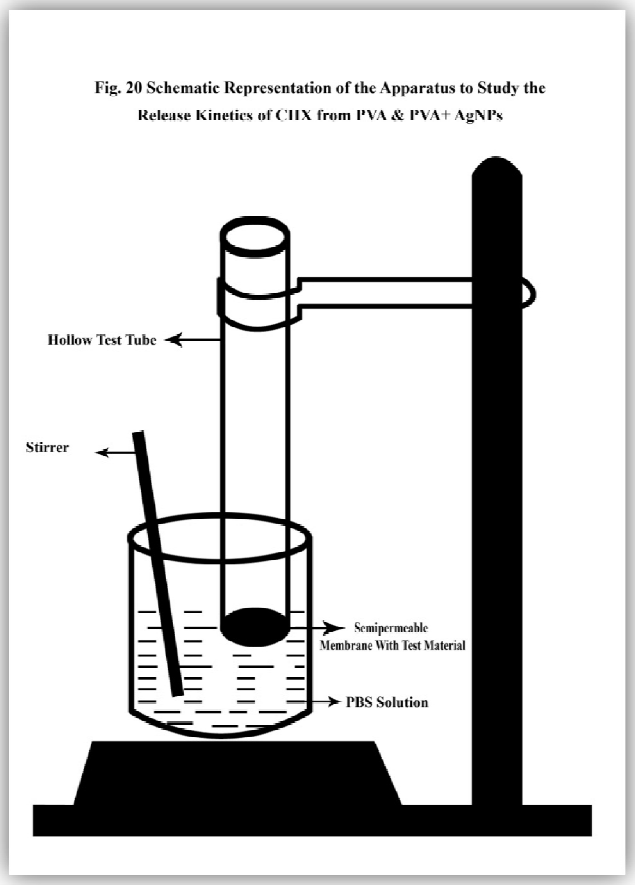
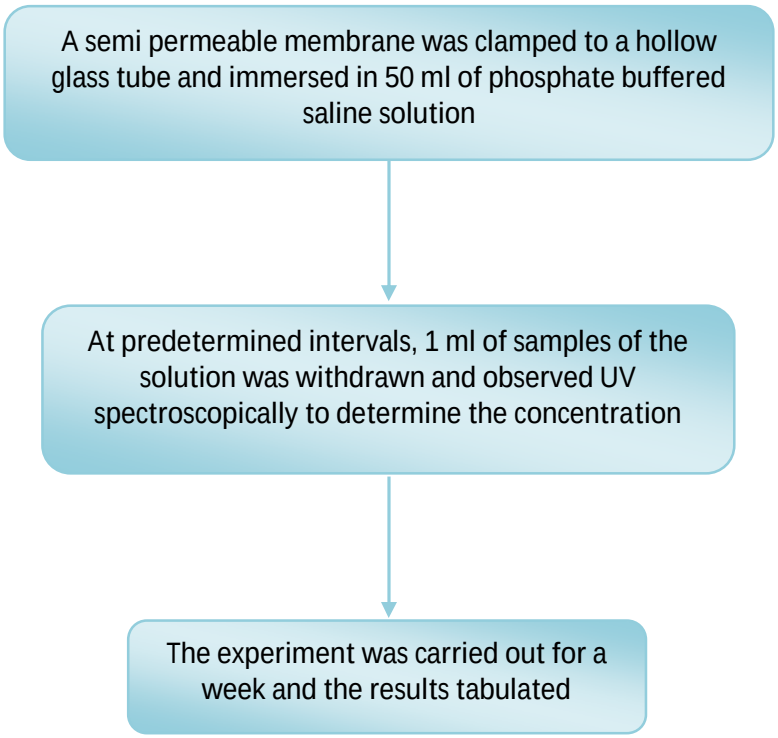


Fig. 20 Schematic Representation of the Apparatus to Study the Release Kinetics of CHX from PVA & PVA+ AgNPs

The cellulose acetate membrane was clamped carefully to one end of the hollow glass tube. 1.2 gm of the test gel i.e PVA + CHX (equivalent to 0.2 gm of the drug) was weighed and obtained from the casting prepared and spread uniformly on the internal aspect of the membrane. The glass tube was submerged in a beaker containing 50 mL of PBS maintained at 37 ± 0.5°C. The PBS solution was

stirred continuously by externally driven Teflon coated magnetic bar. At predetermined time intervals (30 mins, 1 hour, 1 ½ hours….initially and after 10 hours the interval was increased to 24 hours), 1 mL samples of the solutions were withdrawn and replaced with an equal quantity PBS solution. UV Spectrum of the samples was taken. Presence of absorption peak at 254 nm showed the presence of Chlorhexidine in the solution. The drug concentration in the eliquates was thus determined using the UV spectrophotometer. The experiment was done for a period of a week.

Analysis of Drug Release



The experiment was repeated for PVA + AgNPs + Chlorhexidine. Here the amount of gel formed was approximately 0.7 gm. Therefore the whole of the test gel which contains 0.2 gm of Chlorhexidine by dry weight was used for the experiment. Loading of chlorhexidine in both the cases is taken as 0.2 gm to standardize the experiment.

METHODOLOGY FOR MICROBIOLOGICAL STUDIES:

E. faecalis (ATCC 29212) maintained in stock culture in the Department of Microbiology, Madras Medical College was used in this study. Twenty four hours growth of E. faecalis grown on sheep blood agar and Mac Conkey agar plates were suspended in 1ml of peptone broth and incubated for 4 hours at 37°C. The culture suspension was adjusted to match the turbidity equivalent to 0.5 McFarland Standard. This was used as the standardized inoculum for all the procedures.

These inoculate were used to make the lawnculture of the organism using sterile cotton swabs on sheep blood agar. The streaked petridishes were incubated at 37 °C for 24 hrs. When the growth of the organism was confirmed, wells 5 mm deep and 10 mm wide in diameter were then punched in the agar plates with a sterile punch under laminar flow. A total of 20 wells, (1 plate = 2 wells) were prepared. Freshly prepared 50 µml of each test material (which is equal to 25 µml of

the drug) in gel state was placed in the well. Five wells for each test material i.e PVA + 2 % Chlorhexidine digluconate and PVA + AgNPs + 2 % Chlorhexidine digluconate were allocated. Five wells for plain chlorhexidine and 5 wells for saline were allocated.

Group No. 1: 2 % Chlorhexidine digluconate (5 wells)

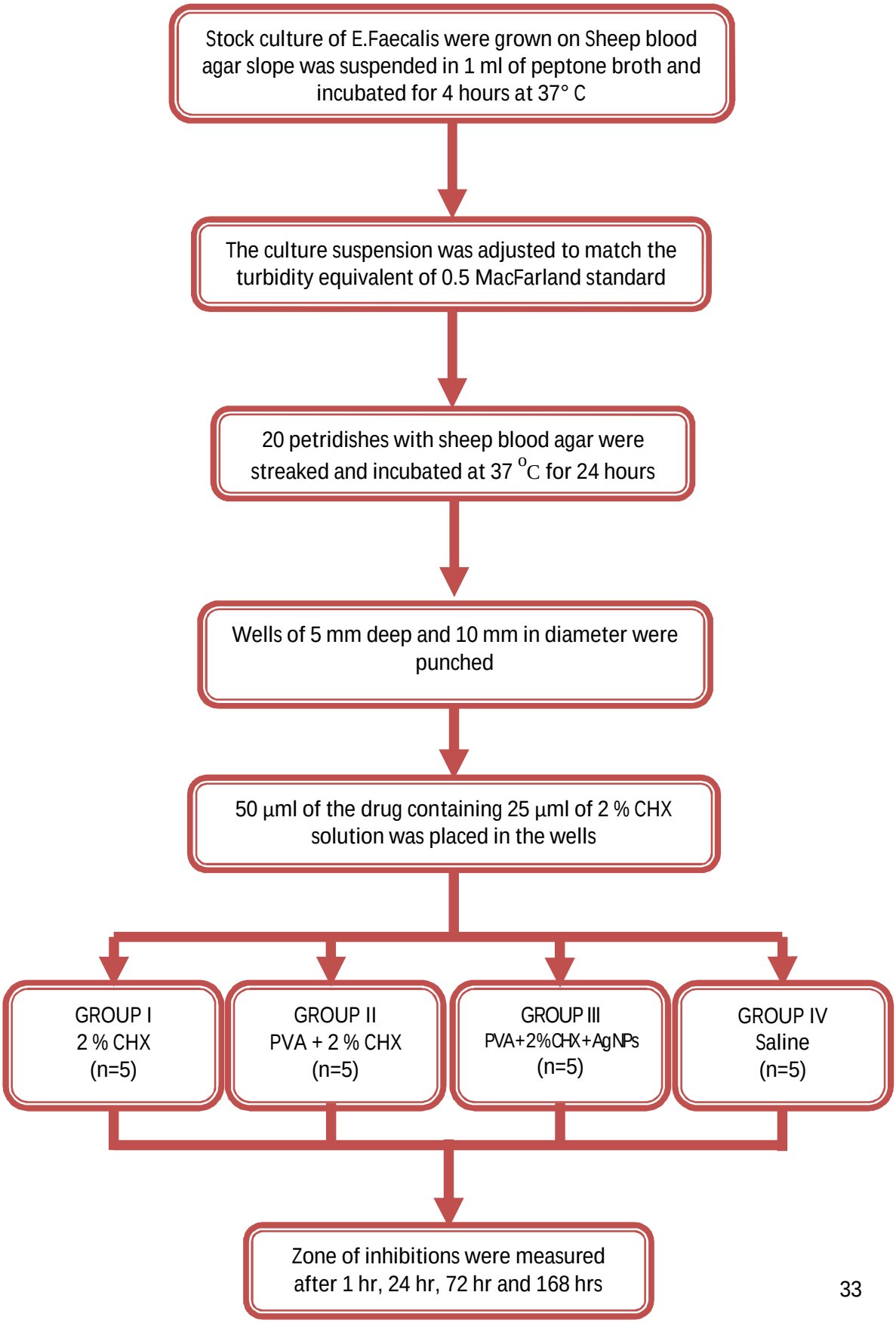
Group No. 2: PVA + 2 % Chlorhexidine digluconate (5 wells)

Group No. 3: PVA + AgNPs + 2 % Chlorhexidine digluconate (5 wells)

Group No. 4: Saline (5 wells)

The agar plates were then incubated at 37°C. The zone of inhibition was measured using a steel ruler from the outer most borders of the wells to the outermost border where the growth begins and multiplied by 2 and was recorded for each material at an interval of 1 hr, 1 day, 3 days and 7 days.

Antimicrobial studies



RESULTS:

UV Spectrophotometric studies:

UV-Vis spectroscopy is routinely used in the quantitative determination of solutions or transition metal ions, highly conjugated organic compounds and biological macromolecules. In this study UV-Vis spectroscopy is used for the indication or the presence of chlorhexidine which produces characteristic peak in the UV visible region around 254 m and Silver nanoparticles which shows a peak at 412 nm.

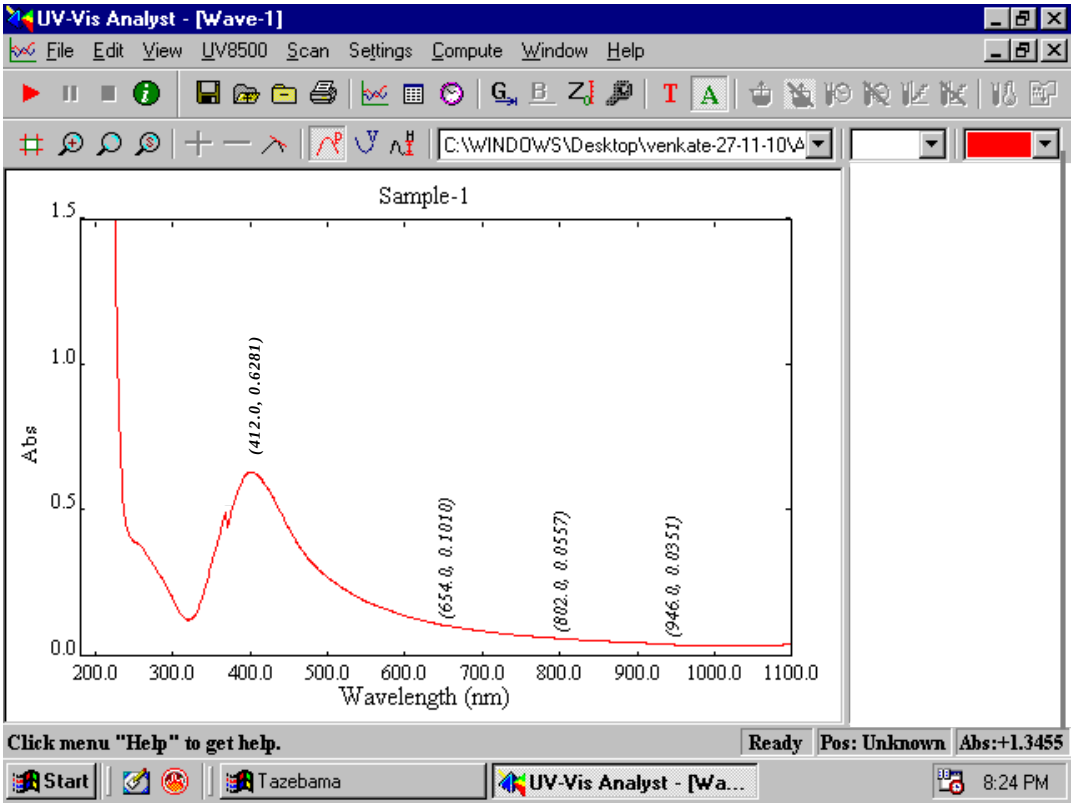


Figure shows UV-Vis spectral changes during the formation of polymer-Ag NPs. A peak at 412 nm is due to the ligand-to-metal charge transfer which appears after heating the mixture of $AgNO_3$ and PVA in water for one hour, indicating that silver ions are completely reduced in the solution. Chlorhexidine produce characteristic peak in the UV-Visible region around 254 nm.

The UV absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution. The wavelengths of absorption peaks can be correlated with the types of bonds in a given molecule and are valuable in determining the functional groups within a molecule.

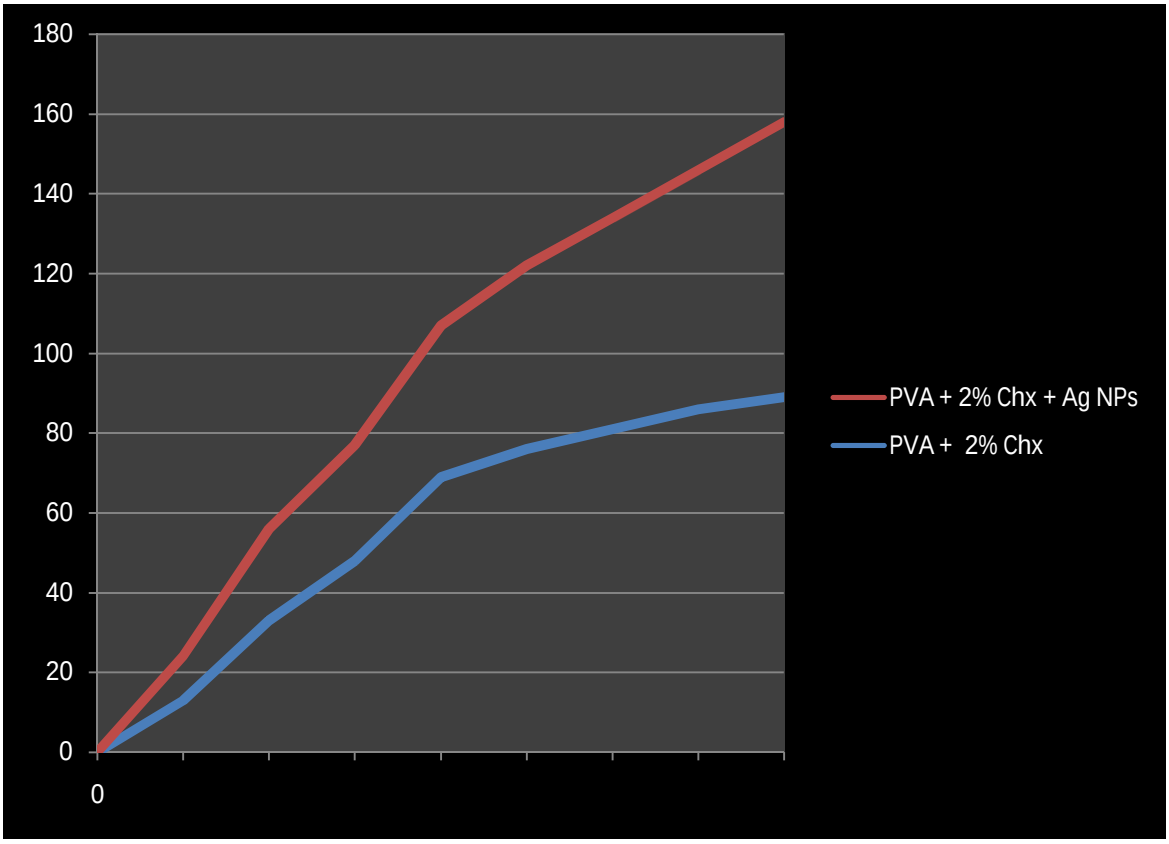
In vitro drug release:

The in vitro release of the drug from Group 2 and Group 3 was studied by the semipermeable membrane diffusion technique. The membrane allows the diffusion of the drug immediately into the receiver compartment containing the PBS solution. One mL samples of the solutions were withdrawn and replaced with an equal quantity of PBS solution. UV Spectrum of the samples was taken. The UV absorbance was converted into concentration to determine the released quantity of chlorhexidine. The

decimals were rounded to the nearest whole numbers. Results are tabulated in tables below.

Time intervals	Cumulative % of CHX release from Group 2 (PVA + 2 % CHX)	Cumulative % of CHX release from Group 3 (PVA + AgNPs + 2 % CHX)
1 hour	13	11
24 hours	33	23
48 hours	48	29
72 hours	69	38
96 hours	76	46
120 hours	81	53
144 hours	86	60
168 hours	89	69

The following is the graphical representation of the comparison of the release kinetics of the two groups:



I. The Results show that the release of CHX from Group 2 is initially high in the first 72 hours followed by a more sustained one, whereas the release from Group 3 is in a sustained manner throughout.

II. The cumulative percentage of CHX release from Group 2 at the end of 1 week is 89 % whereas that for Group 3 is 69 %.

Results of the microbiological studies:

Group 1: 2 % CHX

Group 2: PVA + 2 % CHX

Group 3: PVA + AgNPS + 2 % CHX

Group 4: Saline

Zone of inhibition noted after 1 hour, 1 day, 3 days and 7 days. Each group has 5 reading in all these days. There was no zone of inhibition for Group 5 (Saline).

STATISTICAL ANALYSIS

The statistical analysis was done by using Anova followed by Tukey, HSD test (SPSS 15 version)

The following table shows the mean and standard deviation values of Group I, II and III at 1, 24, 72 and 168 hours

Zone of Inhibition in mm

Hours		Groups					
		Group I		Group II		Group III	
		Mean	SD	Mean	SD	Mean	SD
	1	9.20	1.30	2.40	1.14	.40	.55
	24	31.60	.55	12.00	1.58	16.60	2.07
	72	31.60	.55	25.80	1.30	24.80	1.30
	168	31.60	.55	28.80	1.10	29.00	.71

Group 1: 2 % CHX

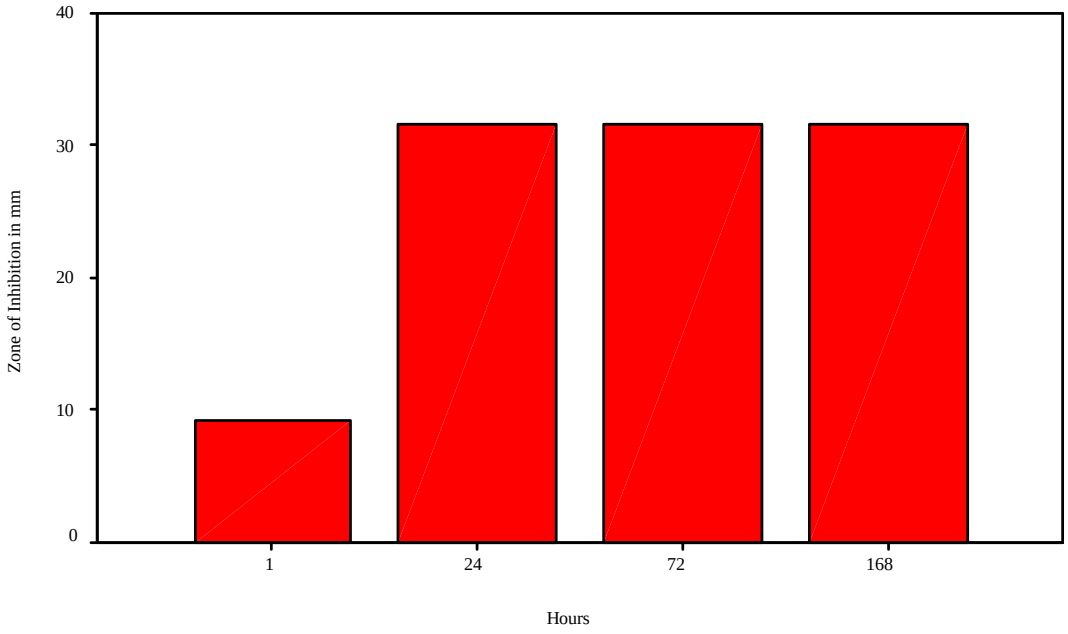
Zone of inhibition measured in mm				
	1 hour	1 day	3 days	7 days
Plate 1	8	32	32	32
Plate 2	9	32	32	32
Plate 3	10	31	31	31
Plate 4	8	32	32	32
Plate 5	11	31	31	31

GROUP I (Oneway)

Zone of Inhibition in mm

Hrs	Mean	Std. Deviation	p Value
1	9.20	1.304	<0.001**
24	31.60	.548	
72	31.60	.548	
168	31.60	.548	

Note: ** denotes significance at 1 % level



Graphical representation of zone of inhibition of Group I after 1 hr, 24 hr, 72 hr and 168 hrs.

Multiple Comparisons

Dependent Variable: Zone of Inhibition in mm

Tukey HSD

(I) Hours	(J) Hours	Mean Difference (I-J)	Std. Error	p- Value	95% Confidence Interval	
					Lower Bound	Upper Bound
1	24	-22.40(*)	.510	.000	-23.86	-20.94
	72	-22.40(*)	.510	.000	-23.86	-20.94
	168	-22.40(*)	.510	.000	-23.86	-20.94
24	1	22.40(*)	.510	.000	20.94	23.86
	72	.00	.510	1.000	-1.46	1.46
	168	.00	.510	1.000	-1.46	1.46
72	1	22.40(*)	.510	.000	20.94	23.86
	24	.00	.510	1.000	-1.46	1.46
	168	.00	.510	1.000	-1.46	1.46
168	1	22.40(*)	.510	.000	20.94	23.86
	24	.00	.510	1.000	-1.46	1.46
	72	.00	.510	1.000	-1.46	1.46

* The mean difference is significant at the .05 level.

1. The difference between the values of the 1st hour compared with the 1st day, 3rd day and 7th day are highly significant with p value ≤ 0.001.
2. But the difference between the values of 1st day, 3rd day and 7th day when compared with each other showed no difference and hence no significance.

Group 2: PVA + 2 % CHX

Zone of inhibition measured in mm				
	1 hour	1 day	3 days	7 days
Plate 1	3	12	24	28
Plate 2	4	14	25	28
Plate 3	2	10	27	30
Plate 4	2	13	27	30
Plate 5	1	11	26	28

GROUP II (Oneway)

Zone of Inhibition in mm

	Mean	Std. Deviation	p value
1	2.40	1.140	<0.001**
24	12.00	1.581	
72	25.80	1.304	
168	28.80	1.095	
Total	17.25	11.002	

Multiple Comparisons

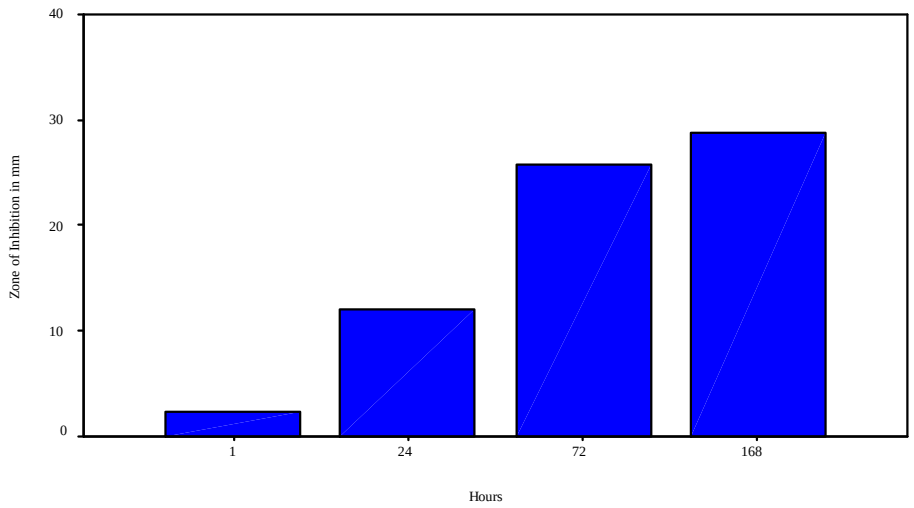
Dependent Variable: Zone of Inhibition in mm

Tukey HSD

(I) Hours	(J) Hours	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	24	-9.60(*)	.819	.000	-11.94	-7.26
	72	-23.40(*)	.819	.000	-25.74	-21.06
	168	-26.40(*)	.819	.000	-28.74	-24.06
24	1	9.60(*)	.819	.000	7.26	11.94
	72	-13.80(*)	.819	.000	-16.14	-11.46
	168	-16.80(*)	.819	.000	-19.14	-14.46
72	1	23.40(*)	.819	.000	21.06	25.74
	24	13.80(*)	.819	.000	11.46	16.14
	168	-3.00(*)	.819	.010	-5.34	-.66
168	1	26.40(*)	.819	.000	24.06	28.74
	24	16.80(*)	.819	.000	14.46	19.14
	72	3.00(*)	.819	.010	.66	5.34

* The mean difference is significant at the .05 level.

1. The difference between the values of the 1st hour and 24 hour compared with 3rd day and 7th day are highly significant with p value ≤ 0.001.
2. But the difference is less when the values of 72 hrs and 168 hrs is compared with p value = 0.010 and hence significant.



Graphical representation of the zone of inhibition for Group II after 1 hr, 24 hr, 72 hr and 168 hr

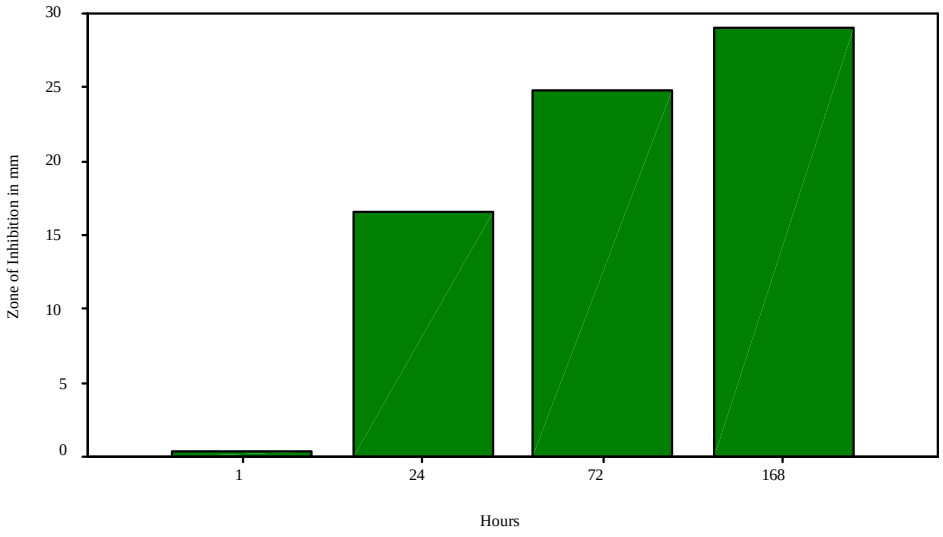
Group 3: PVA +AgNPs + 2 % CHX

	Zone of inhibition measured in mm			
	1 hour	1 day	3 days	7 days
Plate 1	3	12	24	28
Plate 2	4	14	25	28
Plate 3	2	10	27	30
Plate 4	2	13	27	30
Plate 5	1	11	26	28

GROUP III (Oneway)

Zone of Inhibition in mm

	Mean	Std. Deviation	P value
1	.40	.548	<0.001**
24	16.60	2.074	
72	24.80	1.304	
168	29.00	.707	
Total	17.70	11.286	



Graphical representation of the zone of inhibition for Group III after 1 hr, 24 hr, 72 hr and 168 hr

Multiple Comparisons

Dependent Variable: Zone of Inhibition in mm

Tukey HSD

(I) Hours	(J) Hours	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	24	-16.20(*)	.825	.000	-18.56	-13.84
	72	-24.40(*)	.825	.000	-26.76	-22.04
	168	-28.60(*)	.825	.000	-30.96	-26.24
24	1	16.20(*)	.825	.000	13.84	18.56
	72	-8.20(*)	.825	.000	-10.56	-5.84
	168	-12.40(*)	.825	.000	-14.76	-10.04
72	1	24.40(*)	.825	.000	22.04	26.76
	24	8.20(*)	.825	.000	5.84	10.56
	168	-4.20(*)	.825	.001	-6.56	-1.84
168	1	28.60(*)	.825	.000	26.24	30.96
	24	12.40(*)	.825	.000	10.04	14.76
	72	4.20(*)	.825	.001	1.84	6.56

* The mean difference is significant at the .05 level.

The differences between the values of all 4 intervals are highly significant with p value ≤ 0.001.

Intergroup Comparison

Tables

Zone of Inhibition in mm

		Group					
		Group I		Group II		Group III	
		Mean	SD	Mean	SD	Mean	SD
Hours	1	9.20	1.30	2.40	1.14	.40	.55
	24	31.60	.55	12.00	1.58	16.60	2.07
	72	31.60	.55	25.80	1.30	24.80	1.30
	168	31.60	.55	28.80	1.10	29.00	.71

Comparison in 1 hour (Oneway)

Zone of Inhibition in mm

	Mean	Std. Deviation	P value
Group I	9.20	1.304	<0.001**
Group II	2.40	1.140	
Group III	.40	.548	
Total	4.00	4.018	

Multiple Comparisons

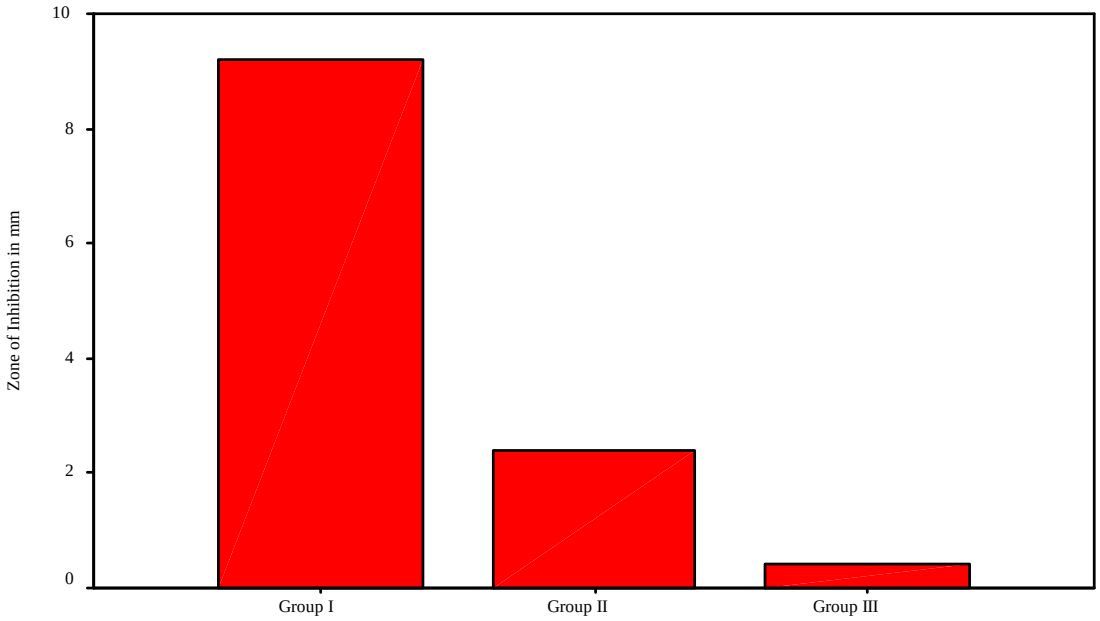
Dependent Variable: Zone of Inhibition in mm

Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group I	Group II	6.80(*)	.663	.000	5.03	8.57
	Group III	8.80(*)	.663	.000	7.03	10.57
Group II	Group I	-6.80(*)	.663	.000	-8.57	-5.03
	Group III	2.00(*)	.663	.027	.23	3.77
Group III	Group I	-8.80(*)	.663	.000	-10.57	-7.03
	Group II	-2.00(*)	.663	.027	-3.77	-.23

* The mean difference is significant at the .05 level.

1. The difference between the values of the Group I compared to Group II and group III after 1 hour are highly significant with p value ≤ 0.001.
2. The difference between the values of Group II and group III is significant with the p value = 0.027.



Graphical representation of the comparison of zone of inhibition of all 3 Groups after 1 hr

Comparison after 24 hours (Oneway)

Zone of Inhibition in mm

	Mean	Std. Deviation	P - Value
Group I	31.60	.548	<0.001**
Group II	12.00	1.581	
Group III	16.60	2.074	
Total	20.07	8.779	

Multiple Comparisons

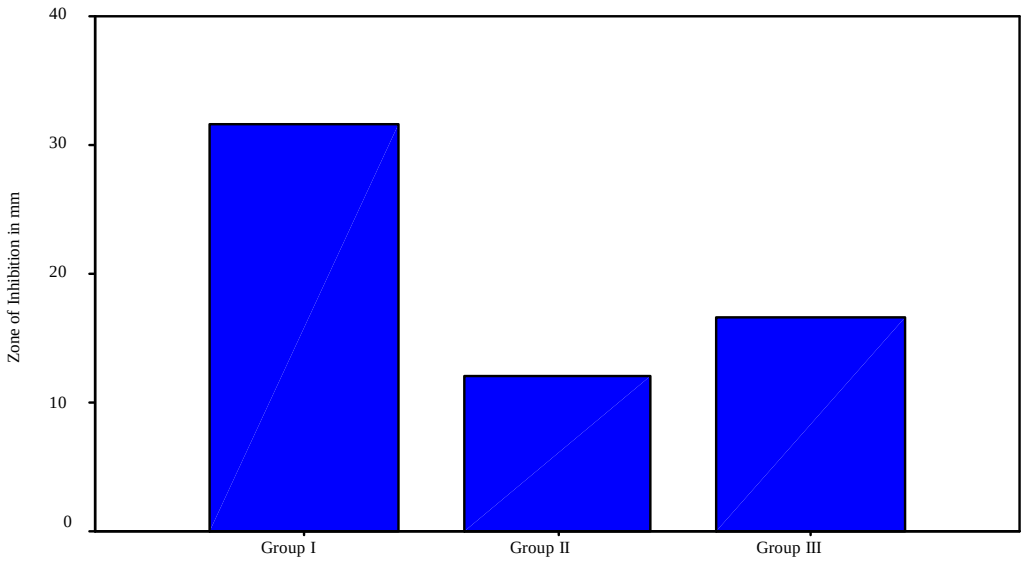
Dependent Variable: Zone of Inhibition in mm

Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group I	Group II	19.60(*)	.973	.000	17.00	22.20
	Group III	15.00(*)	.973	.000	12.40	17.60
Group II	Group I	-19.60(*)	.973	.000	-22.20	-17.00
	Group III	-4.60(*)	.973	.001	-7.20	-2.00
Group III	Group I	-15.00(*)	.973	.000	-17.60	-12.40
	Group II	4.60(*)	.973	.001	2.00	7.20

* The mean difference is significant at the .05 level.

The difference between the values of Group I, II and III when compared to each other are highly significant.



Graphical representation of the comparison of zone of inhibition of all 3 Groups after 24 hr

Comparison after 72 hours (Oneway)

Zone of Inhibition in mm

	N	Mean	Std. Deviation	p - Value
Group I	5	31.60	.548	<0.001**
Group II	5	25.80	1.304	
Group III	5	24.80	1.304	
Total	15	27.40	3.269	

Multiple Comparisons

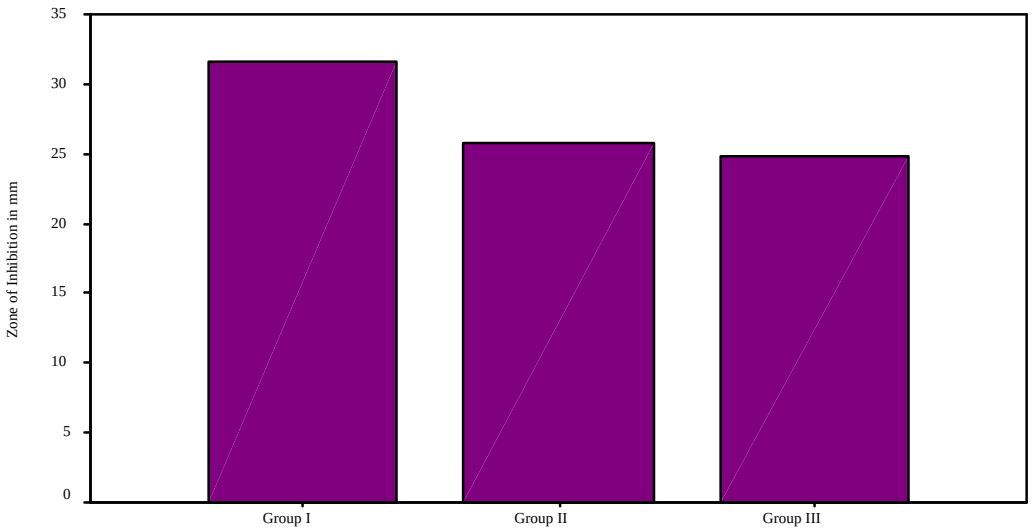
Dependent Variable: Zone of Inhibition in mm

Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group I	Group II	5.80(*)	.702	.000	3.93	7.67
	Group III	6.80(*)	.702	.000	4.93	8.67
Group II	Group I	-5.80(*)	.702	.000	-7.67	-3.93
	Group III	1.00	.702	.360	-.87	2.87
Group III	Group I	-6.80(*)	.702	.000	-8.67	-4.93
	Group II	-1.00	.702	.360	-2.87	.87

* The mean difference is significant at the .05 level.

1. The difference between the values of the Group I when compared with group II and Group III are highly significant with p value ≤ 0.001.
2. The difference between the values of Group II and Group III compared with each other showed no difference and hence no significance.



Graphical representation of the comparison of zone of inhibition of all 3 Groups after 72 hours

Comparison after 168 hours (Oneway)

Zone of Inhibition in mm

	Mean	Std. Deviation	p - Value
Group I	31.60	.548	<0.001**
Group II	28.80	1.095	
Group III	29.00	.707	

Multiple Comparisons

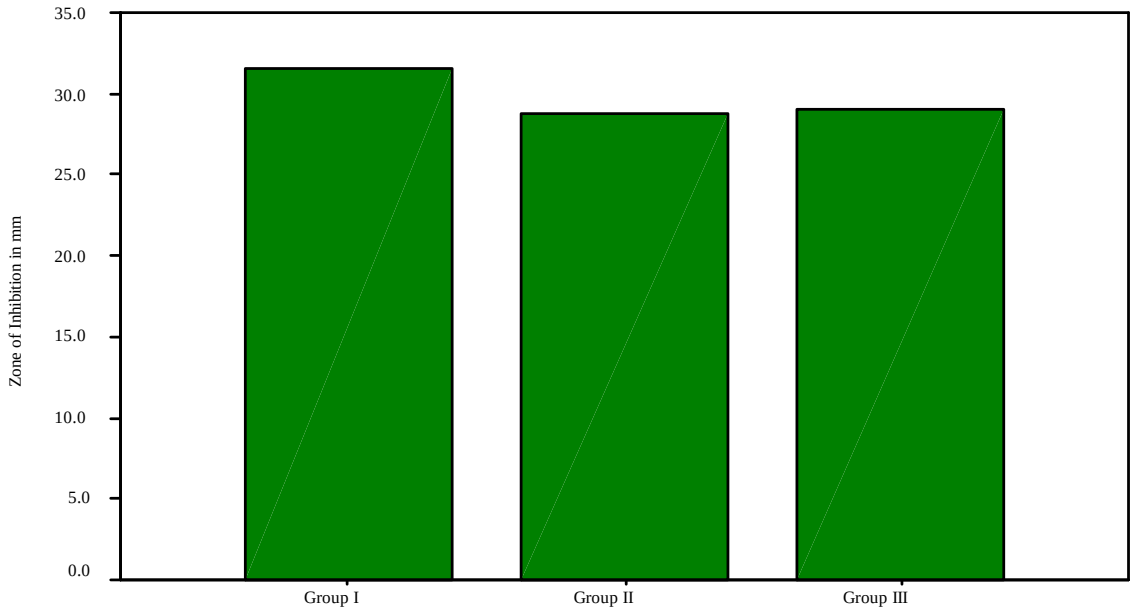
Dependent Variable: Zone of Inhibition in mm

Tukey HSD

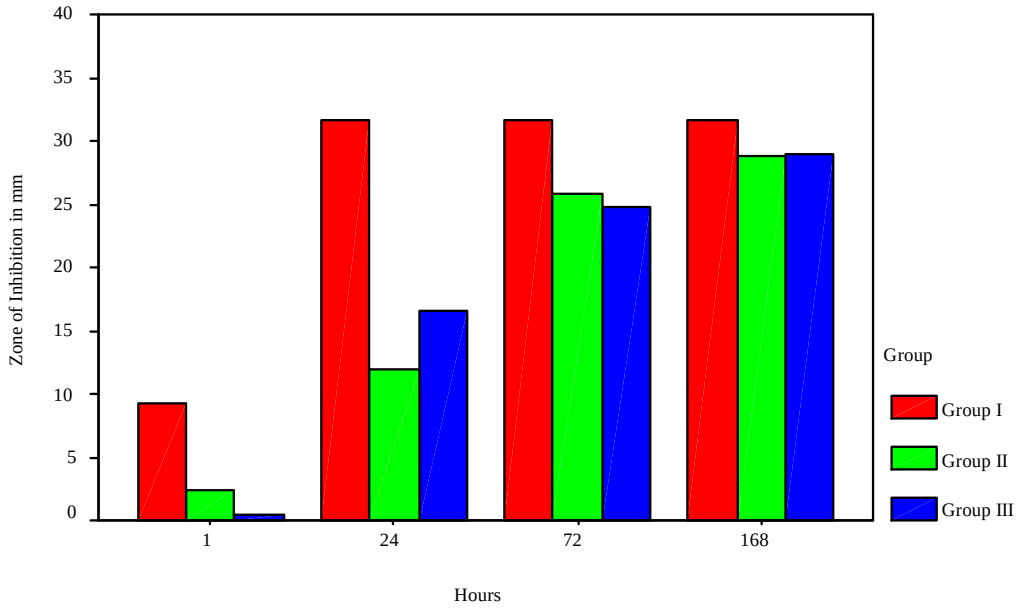
(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group I	Group II	2.80(*)	.516	.000	1.42	4.18
	Group III	2.60(*)	.516	.001	1.22	3.98
Group II	Group I	-2.80(*)	.516	.000	-4.18	-1.42
	Group III	-.20	.516	.921	-1.58	1.18
Group III	Group I	-2.60(*)	.516	.001	-3.98	-1.22
	Group II	.20	.516	.921	-1.18	1.58

* The mean difference is significant at the .05 level.

1. The difference between the values of the Group I when compared with group II and Group III are highly significant with p value ≤ 0.001.
2. The difference between the values of Group II and Group III compared with each other showed no difference and hence no significance.



Graphical representation of the comparison of zone of inhibition of all 3 Groups after 72 hours



Comparison of Zones of inhibition for all the three groups at various time intervals

DISCUSSION

Microorganisms play a fundamental role in the etiology of pulp and periapical diseases. Their control and elimination are important during endodontic treatment[24]. E. faecalis is probably the species that can best adapt to and tolerate the ecologically demanding conditions in the filled root canals. It is resistant to various antimicrobials. It is considered as a 'star' in post-treatment disease[19]. Because of this the development of effective therapeutic modalities against E. faecalis is desirable. Hence E. faecalis was chosen for the inoculum in this study. In addition, E. faecalis is relatively easy to culture and it has been used successfully in most studies with the original model[2,16,29,33].

Various modalities are attempted to eliminate residual bacteria from the root canal, which includes high end technologies as with LASERS, PAD (Photo Activated Disinfection) and ANILAD (Advanced Non invasive Light Activated Disinfection). Even though these show promising results, its applicability to all clinical settings and private practice may be difficult because of the exorbitant cost involved. A cost effective way to deal with the same situation is using intra canal medicaments. The use of an intra canal medicament helps in the elimination of bacteria that remains even after

cleaning and shaping, thereby providing an environment conducive for periapical tissue repair[6].

Many medicaments were used as intracanal dressings in the past decades and according to their chemical basis, generally fall into the following categories: phenolic derivatives (eugenol, camphorated paramonochlorophenol, camphorated phenol, metacresyl acetate, beechwood creosote), aldehydes (formocresol), halides (iodine–potassium iodide), calcium hydroxide, antibiotics, and various combinations.

The most popular intracanal medicament in use currently is calcium hydroxide. However, various studies confirmed the resistance of E. faecalis to Calcium hydroxide. Various newer medicaments are also tried nowadays, as Bioactive glass and Octanidine. 2% Chlorhexidine (CHX) has been found to be highly active against E. faecalis[8]. CHX seems to act by adsorbing on to the cell wall of microorganisms and causing the leakage of intracellular components. In addition, it is retained by the dentinal hard tissues and thus has a substantive antimicrobial action[2,16,29]. The cationic properties of chlorhexidine allow it to be absorbed on to hydroxyapatite[32]. It was also suggested as an effective irrigant to prevent root canal re-infection due to coronal leakage[22]. However, to achieve long-term substantive anti microbial effect, the infected root dentin must be exposed to CHX for a longer time

than that afforded by irrigation[16,32]. It therefore seems reasonable to assume that chlorhexidine has a potential role as an effective intracanal medicament.

CHX is an effective intracanal irrigation compared with 5% Sodium hypochlorite[22]. However, the relatively short exposure time (a few minutes at the most) in the root canal during irrigation does not allow the medication to apply its full antibacterial properties. Thus, a large amount of bacteria remained in the dentinal tubules. The limited antibacterial effect of CHX irrgation is due to the fact that during the first hour of irrigation the dentin absorbs the medication[61] and it is only after the saturation point after the first hour that the antibacterial capability of CHX increases with time. Therefore the sustained release mechanisms provide an optimal condition for a maximal antibacterial effect of the medication[73].

A major research thrust in the pharmaceutical and chemical industry is the development of controlled release systems for drugs and bioactive agents. Many of these delivery systems in use and under development consist of a drug dispersed within a polymeric carrier. These carriers are designed to release the drugs in a controlled fashion for times ranging from minutes to years.

Thus, polymers have gained in importance in the pharmaceutical industry as both drug encapsulants and vehicles of drug carriage. They have

the property of sustained/retarded release, and/or selective delivery of the drug to the targeted organs. Advantages of sustained release systems thus include: delivery to the required site; delivery at the required rate; fewer applications; and also economic advantages by virtue of more efficient dosage.

Sustained release of chlorhexidine has been studied from various polymeric media like gelatin[4], collagen[1], ethyl cellulose[10], resins[7,23], fibres[51], Glass ionomer Cement[40], chitosan[37,56], PMMA Polymethyl methacrylate[33] and PLGA poly(lactic-co-glycolic acid)[51]. Most of these sustained release systems have found profound application in periodontology wherein they are used inside the infected periodontal pockets.

There are considerable studies in the field of endodontics regarding the sustained release of Chlorhexidine. . The ideal controlled release drug device for endodontic usage should have the following characteristics. It should not degrade inside the root canal and it should be easily inserted into and removed from the root canal. In addition, the drug should be released continuously for a controlled time period.

Friedman & Steinberg (1989) first introduced a degradable sustain release device (SRD) to dentistry which contained chlorhexidine gluconate as the active agent. It was later evaluated for endodontic use by Heling et al

in 1990. The matrix of this SRD is composed of a crosslinked protein with chlorhexidine gluconate embedded as the active agent.The rate of release of chlorhexidine gluconate from the device depends on the following factors[16]:

I. The initial amount of chlorhexidine loaded into the film.

II. There is an inverse relationship between the crosslink density and the rate of Chlorhexidine release from the film. The higher the crosslink density, the more prolonged the antibacterial activity.

III. The amount of liquid in the root canal that will be absorbed by the device.

IV. The number of bacteria which enhance the biological enzymatic degradation mechanism.

In a study conducted by **Spa°ngberg** and Doug-Youn Lee in 2005[37], a needle shaped controlled release prototypes, first developed by Huang et al[17] were used. The prototypes (paper points) were either coated (with polymers) or non-coated and loaded with chlorhexidine and its release was studied. They demonstrated that the releasing rate of the prototype with non-coated formulation was very fast (CHX was released within 2 hours). In contrast, the release rate of the prototypes with coated formulations was far more controlled. The polymer coatings used were chitosan, PLGA and PMMA.

Chitosan was more sensitive to water and easily swollen with water and ruptured. This resulted in faster release of CHX from the chitosan-coated prototype compared to the PLGA- and PMMA-coated groups. The CHX loaded in the paper point was released through the surface pores on the coated polymer layer. The pore size of PLGA-coated group was larger than that of PMMA-coated group and the release rate of CHX from the latter group was lower than that of the former group. Thus, drug release can be controlled by controlling the pore size of the coated polymer.

Shaul Lin et al[61] used Activ Point containing CHX as intracanal medicament. The advantages which he quoted were: Gutta-percha points containing CHX are IS0 sizes and radiopaque, the compatible IS0 size Activ Point is accurately applied to the working length after irrgation with saline solution. It is not necessary to dry the canals because the gutta-percha points release the CHX in the presence of the solution. The accurate application of the gutta-percha points to the working length ensures antibacterial activity along the root canal and prevents extension of the medication into the periradicular tissues. Removal of the gutta-percha points after 7 or 14 days is easy and eliminates the possibility of paste remnants on the dentinal walls, which could disturb the adhesive properties of the intracanal sealer. They concluded that Activ Point seems to be an effective intracanal medication

with high penetration ability to dentinal tubules and strong antibacterial properties.

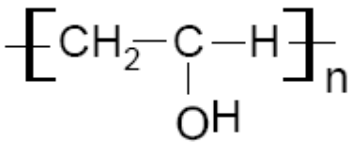
In a more recent study by **Yoon Lee et al 2008**[73] evaluation of a similar PCRD (Polymeric chlorhexidine control release device) coated with Polymethyl methacrylate and loaded with high concentration of CHX as an intracanal medicament was done and proved it to be superior than Calcium hydroxide and 0.2% chlorhexidine.

The above mentioned studies have been carried out for the in vitro application of the sustained release mechanism of Chlorhexdine which have already been proved. In this study a well known polymer in the field of polymer science and pharmaceutical industry known as Polyvinyl alcohol (PVA) has been used to evaluate the control release of the drug of our choice which is chlorhexidine digluconate. PVA has been chosen in this study because it is a hydrophilic polymer with unique properties of absorbing water and forming hydrogels and therefore is extensively used in sustained release applications[20].

PVA possesses a variety of advantageous chemical and physical properties like chemical resistance, high melting point and biological compatibility and represents an inexpensive, non-toxic material which is

commercially available in a broad range of degrees of polymerisation and hydrolysis[59].

The chemical structure of Polyvinyl alcohol is as follows with a glass transition temperature of 85 ^{0}C.



Polyvinyl alcohol Tg = 85^{0}C

We have employed the hydrogel forming property of PVA in our study for the sustained release of Chlorhexidine. One major class of polymers that has been identified for use in controlled release applications is hydrogels. Hydrogels are three-dimensional, water-swollen structures composed of mainly hydrophilic homopolymers or copolymers[44]. The crosslinked three dimensional polymeric arrays can imbibe large quantities of water. In the hydrated state these materials are moist, pliable and can conform to irregular surfaces[11]. These materials are for the most part insoluble due to the presence of chemical or physical crosslinks. The physical crosslinks can be entanglements, crystallites or weak associations such as van der Waals forces or hydrogen bonds. The crosslinks provide the network structure and physical integrity.

Hydrogels are classified in a number of ways. They can be neutral or ionic based on the nature of the side groups. They can also be classified based on the network morphology as amorphous, semicrystalline, hydrogen-bonded structures, supermolecular structures and hydrocolloidal aggregates. Additionally, in terms of their network structures, hydrogels can be classified as macroporous, microporous, or nonporous.

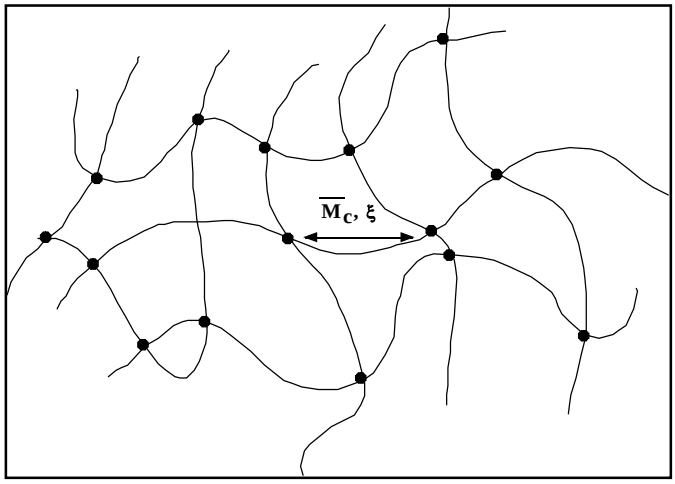
Because of their wide range of properties, hydrogels have been considered in drug delivery applications for over 30 years. Some of the application of hydrogels include[55];

- Wound healing wherein antibacterial drugs like chlorhexidine and coagulants can be formulated with gelatin and PVA hydrogel,
- Controlled delivery of ibuprofen to colon has been achieved through hydrogel of guargum cross linked with glutaraldehyde,
- In topical delivey like desonide, cotrimazole, in ocular delivery of pilocarpine, timolol, diclofenac, pheneramine maleate.

Two of the most important characteristics in evaluating the ability of a polymeric gel to function in a particular controlled release application are the network permeability and the swelling behavior. The permeability and swelling behavior of hydrogels are strongly dependent on the chemical nature of the polymer(s) composing the gel as well as the structure and

morphology of the network. As a result, there are different mechanisms that control the release of drugs from hydrogel-based delivery devices and these characteristics allow these systems to provide many different release profiles to match desirable release profiles.

A schematic representation of a hydrogel structure is given below (Anthony M.Lowman)



Schematic representation of the cross-linked structure of a hydrogels. $\overline{M}_C$ is the molecular weight of the polymer chains between crosslinks and ξ is the network mesh size.

The transport or release of a drug through a polymeric controlled release device can be described by classical Fickian diffusion theory. This theory assumes that the governing factor for drug transport in the gels is ordinary diffusion.

The structure and morphology of a polymer network will significantly affect the ability of a drug to diffuse through a hydrogel. For all types of release systems, the diffusion coefficient of solutes in the polymer is dependent on a number of factors such as the structure and pore size of the network, the polymer composition, the water content and the nature and size of the solute. Macroporous hydrogels have large pores, usually between 0.1 and 1 μm. Microporous Hydrogels have pore sizes between 100 and 1000 Å.

Polyvinyl alcohol hydrogels have been used for controlled drug delivery system for many drugs like propanalol, atenalol, indomethacin, phenylpropanalamine[36], theophylline[20] etc. There have been very few studies in the past which have dealt with PVA hydrogel for the controlled release of Chlorhexidine.

The hydrogels formed by PVA is quite hydrophilic system, it releases drug with relatively high rates. Therefore, to prolong the drug release from such system, its molecular structure should be modified. Usually this is done by cross linking and reducing the macromolecular mesh size available for drug diffusion[20]. The crosslinking can be chemical or physical. Chemical crosslinking includes addition of cross linkers like Gluteraldehyde, glycerol etc under acidic condtions[20].

In our study we have carried out the physical cross linking by heating the polymer above its glass transition temperature Tg (85°C). In such conditions the polymer chains gain enough mobility to align themselves and therewith to migrate into more ordered and energetically favourable conformations[59]. Also strong hydrogen bonding between neighbouring polymer chains holds the molecules in a regular structure and forms ordered regions (crystallites) beside the amorphous polymer matrix[58].

Gay et al[11] has used PVA for the slow release of chlorhexidine diphosphanilate for wound dressings and had studied the results for a period of 1week. He had taken PVA of different grades (523, 425, 350) depending on the degree of hydrolysis and other parameters and had shown that the release was complete for PVA 523 in 40 hrs whereas only 50 % of the drug was released from PVA 425 and 350 after 40 hrs and the release continued after that. He concluded that greater is the degree of hydrolysis, more is the solubility in water and rate of release is faster. PVA 523 has a degree of hydrolysis of 87-89 % whereas for PVA 425 it is 95.6 – 96.5 % and for PVA 350 it is 98-98.8%. In this study PVA with a degree of hydrolysis of 97.5-99.5 % hydrolysis has been used.

Importantly, two groups have been compared in this study one is Group 2 wherein release of CHX from Polyvinl alcohol and the other is

Group 3 wherein effect of Silver nanoparticles (AgNPs) on the release of CHX from Polyvinyl alcohol and the antibacterial activity are seen. The results of the release study showed that nearly 89 % of the drug was released in a period of a week from plain PVA (Group 2) whereas only 69 % of the drug was released from PVA with AgNPs (Group 3). The release continued for the two groups thereafter. For group 2 the release was high in the first 72 hrs followed by a more sustained one.

The reason for the initial burst may be due to non uniform or heterogenous drug distribution[74] and the surface bound drug particles which are first released followed by the release of more deeply entrapped ones in the meshwork. The minimal disadvantage of burst release is that it leads to a loss in treatment efficacy, as drug is lost in an uncontrolled and unpredictable pattern[72]. For group 3 the initial burst was not very significant and the release was approximately uniform. The slower drug release from this group may be attributed to the presence of silver nanoparticles which alter the drug release from a polymer depending on the size. The nanoparticle size was associated with change in drug release kinetics. The smaller size nanoparticles prepared with lower amount of polymer exhibited higher drug release rate, this is may be due to the increased nanoparticle surface resulting in larger drug fraction exposed to the dissolution medium

and also the higher amount of drug loading. The larger size nanoparticles prepared with higher amount of Polymer exhibited lower drug release rates, this may be due to the decreased nanoparticle surface resulting in smaller drug fraction exposed to the dissolution medium and also the lower amount of drug loading[38]. Therefore, further studies are required wherein the nanoparticle size should be standardized for better control of sustained drug release.

The agar diffusion test was employed in this study to determine the antimicrobial efficacy of the test medicaments. The agar diffusion test is generally an accepted method to test the antimicrobial activity of endodontic medicaments and irrigating solutions. This test is a standardized one making it reproducible and simple to perform and relatively inexpensive.

The results of the agar diffusion test which was done at intervals of 1 hr, 1 day 3 days and 7 days showed that compared to Group 1 (2% CHX), Group 2 and 3 showed slower release of the drug and hence slowly developing zone of inhibition. Within 1 hour Group 1 attained its maximum zone of inhibition and there was no change in it after 7 days also. For Group 2 the release after 1 hour was not very significant but it increased and the zone of inhibition after 3 days was significantly larger. Thereafter the

increase was at a lower rate. At the end of seven days when compared to Group 1, the zone of inhibition of Group 2 was smaller by an average of 3 mm.

In Group 3, the zone of inhibition was negligible in the 1st hour, it slowly increased in size and after 1 day it attained size greater than group 2. The zone of inhibition further increased and the average of the readings at the end of both 3rd and 7th day was mildly larger that group 2. This is not in accordance with the release studies because, in the microbiological studies, the effect of the silver nanoparticles is synergistic with Chlorhexidne against E. Faecalis. The antibacterial activity of silver nanoparticles against E. Faecalis has been proved[70].

Some factors such as the pH of the substrate, incubation period, toxicity, and sensitivity and diffusion capacity of the drug may have an impact on the antimicrobial activity of the test materials in the plates. Also, this is the first time that a combination of PVA, AgNPs and chlorhexidine has been used in the agar diffusion test. The components may have effect on the diffusion of chlorhexidine.

This is a very preliminary study to prove a point that chlorhexidine can be slowly released from biocompatible hydrogels like PVA. Further studies are required to find out the methods of better manipulation, further improvement of the polymers for better sustainment of the drug, modifications in the concentration of the drug used for better efficacy and the results of application of this sustained release system and their efficacy in the more complicated in vivo conditions.

SUMMARY

Endodontic treatment is targeted towards the reduction and elimination of microorganisms from the root canal system. Intra canal medicaments can aid in achieving this objective. 2 % CHX is being used as a successful irrigant against many pathogens in endodontics. The drawback with the irrigant is that the solution is in contact with the root canal dentin for a very short period of time. Hence, the use of this drug as an intra canal medicament with a sustained drug releasing medium would be more efficient in the elimination of the pathogens.

This study analyses the release mechanism of 2 % CHX from a polymeric medium for a prolonged period of time (1 week) so that it can be used as an intra canal medicament to get enhanced results. A well known polymer used in the pharmaceutical industry as a drug carrier called polyvinyl alcohol with the property of forming hydrogel is used here. 2 % CHX is incorporated in this hydrogel matrix (Group 2) and the slow release of this drug is studied over a period of a week.

In Group 3 the above mentioned combination along with the effect of silver nanoparticles is studied and the results are compared with that of the

plain chlorhexidine (Group 1). Group 2 with PVA and 2 % CHX showed 89 % of the drug release at the 168th hour (7th day). Group 3 with PVA and AgNPs and 2 % CHX showed 69 % of the drug release at the end of 168th hour.

Agar diffusion method was used to compare the zone of inhibition produced by the slow release of the drug against E faecalis over a period of a week. The following groups were tested.

Group 1: 2 % CHX

Group 2: PVA + 2 % CHX

Group 3: PVA + AgNPS + 2 % CHX

Group 4: Saline

The results were monitored on 1st hour, 1st day, 3rd day and 7th day. Group 1 showed maximum zone of inhibition within 24 hours, whereas the zone of inhibition slowly increased over a period of a week for the 2nd and the 3rd group showing the slow drug release. Group 4 with saline showed no zone of inhibition.

CONCLUSION

Chlorhexidine is widely used in the field of endodontics as it is highly effective against many root canal pathogens including E faecalis. The result of the present study shows that the efficacy of chlorhexidine can be further enhanced by incorporating the drug in a sustained release medium as it makes the availability of the drug throughout the course of the treatment. The usage of polyvinyl alcohol as a carrier of the drug along with the synergistic properties of silver nanoparticles, opens a new avenue of trying a biocompatible polymer in endodontics as an intra canal medicament to act against the microorganisms in the root canal system for an extended period of time and aid in the success of endodontic treatment.

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